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A STUDY OF LACTOSE-FERMENTING YEASTS PRESENT IN "YEASTY" CREAM

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A Study of Lactose-fermenting Yeasts Present in "Yeasty" Cream

By B. W. Hammer and W. A. Cordes

The shipment for considerable distances of cream to be made into butter, either from receiving stations or from individual farmers, is a common practice in many sections. Much of this cream is sent without refrigeration and undergoes extensive changes as a result of the activity of the organisms contained. The type of fermentation that occurs is influenced to a considerable extent by the types of organisms present, but other factors, the most important of which is apparently the temperature, cause a significant effect. One of the important fermentations occurring in cream during the warm months is the "yeasty" or "foamy" fermentation.

Typical "yeasty" or "foamy" cream has a definite yeasty odor and shows much gas formation. Cans in transit often have part of the cream forced out by the gas development. In the receiving rooms of the large creameries the can covers are commonly thrown violently when the seals are broken and the cream then foams out of the cans. The actual loss of cream is of importance, but in addition to this there is a deterioration in the quality of the cream as a result of the development of the responsible organisms. "Yeasty" cream cannot be made into the best quality of butter, since the characteristic odor and flavor are carried over into the finished product. The presence of acid apparently has no restraining influence on the development of the typical "foamy" condition. The "yeasty" fermentation of cream is accordingly of importance from the standpoint of the transportation of sour cream.

From a practical consideration, the object of a study of the "yeasty" fermentation is its elimination or control. Accomplishment of this object is dependent upon knowledge, first, whether or not the fermentation is always due to the same organism, and second, whether or not the organisms causing this change are characteristic of "yeasty" cream alone, or are present in cream, milk and other materials that do not show this condition. The work herein reported was carried out in the hope of supplying information on these two points.

HISTORICAL

The odor of "yeasty" or "foamy" cream is very suggestive of yeasts and the prevalent idea, even among those who have given the subject no particular attention, is that yeasts are the

responsible organisms. Since gas is evolved in such quantities in the typical fermentation, it is quite logical to assume that the yeasts are lactose-fermenters.

In a study of "yeasty" cream, Hunter (11) examined numerous samples from different parts of Kansas and found the predominating type of organism to be a lactose-fermenting yeast. He says: "Raw or sterile cream inoculated with a pure culture of the yeast shows typical foaming characteristics."

Lactose-fermenting yeasts have been found to be the cause of an abnormal condition in cheese in a number of instances. In his studies on Italian cheese, Bochicchio (3) found a yeast capable of fermenting lactose with the production of CO_2, responsible for the undesirable swelling. He proposed the name *Lactomyces inflans casei-grana* for the organism. It coagulated milk in a few days and partial proteolysis followed. Hard cheese made from milk inoculated with a culture of the yeast developed a "huffed" appearance, with large holes in the surface layers. In whey, the yeast produced a foaming, pleasant-tasting beverage.

Harrison (8) described under the name of *Torula amara* a lactose-fermenting yeast causing a bitter taste in milk and cheese. The bitter taste was detected in milk after five or six hours at 37° C., and after fourteen hours the taste and smell were disagreeable and strong; after ten days the milk was slightly thickened and slightly acid. The organism was found associated with the leaves of a certain species of maple near which the milk cans of a factory having trouble with the bitterness were kept.

Russell and Hastings (18) found a lactose-fermenting yeast as the cause of a gassy fermentation in Swiss cheese. They found that in the factory experiencing the trouble, ideal conditions existed for the growth of the organism, since the whey was held in wooden vats for twenty-four hours to allow creaming of the fat. The cream skimmed from these vats had a decidedly yeasty odor and contained a lactose-fermenting yeast, whose development was favored by the strongly acid reaction of the whey. During the season over two tons of cheese were utterly ruined and a loss of over $2000 incurred by the one factory. In a later investigation Hastings (9) found that lactose-fermenting yeasts were widely distributed in such products as butter, milk, cheese, whey, and rennet.

In addition to the lactose-fermenting yeasts already mentioned as responsible for rather extensive abnormal conditions in dairy products, many others have been isolated from various sources and described. Dombrowski (4) has recently reviewed these in an extensive way and the following is in part taken from his report.

The first budding fungus able to break up lactose into alcohol and CO_2 was found in milk in 1887 by Duclaux (5). Its small size distinguished it from the yeasts known to the fermentation industries.

In 1889 Adametz (1) isolated from milk and described a new lactose-fermenting yeast which he named *Saccharomyces lactis*. This organism and *Saccharomyces tyrocola*, which was repeatedly encountered in Edam cheese by Beyerinck (2), have been exhaustively studied by Heinze and Cohn (10). *Sacch. tyrocola* was found to produce a much quicker and more energetic fermentation in milk, to prefer a lower temperature, and to be less resistant to heat than *Sacch. lactis*. Neither of the organisms was shown to produce spores, and Heinze and Cohn suggested that the names be changed to *Torula lactis* Adametz and *Torula tyrocola* Beyerinck. The latter organism and *Torula kefir* (isolated from kefir grains) were supposed, according to Beyerinck, to hydrolize lactose and sucrose, but not maltose.

Heinze and Cohn observed that most of thê lactose-fermenting yeasts were torulae, and that several organisms belonging to the mold fungi also possessed the ability to ferment lactose. They referred to two yeast-like mold fungi which Lindner called lactose-fermenters, altho the lactose fermenting ability of one of these forms, *Monilia variabilis*, had not been determined absolutely. This yeast-like organism was first found by Lindner on damp white bread. The second organism Lindner called *Sachsia suaveolus;* it was supposed to produce an exceptional "bouquet" in wort.

Macé (14) demonstrated that the French soft cheeses very commonly contain lactose-fermenting yeasts. He found in all eleven lactose-fermenting yeasts, ten of which were torulae, in eight out of thirteen samples of this type of cheese.

Two lactose-fermenting yeasts were found by Adametz and Winkler (20) in Olmützer "quargel" cheese; one of these organisms, which gave a yellow-green fluorescent pigment on gelatine, produced CO_2 from sugars, but no alcohol.

Nikolajewa (15) isolated two torulae from kefir grains, one of which fermented lactose, sucrose, and dextrose. These forms were supposed to produce the coat of the grain.

Among the lactose-fermenting yeasts producing spores (true Saccharomycetes) the following are of importance:

(1) A yeast isolated by Grotenfelt (6) from a sample of Finland milk was considered by Hansen to be a true Saccharomycete and was named *Saccharomyces acidi lactici* by Grotenfelt. This form produced in sterile milk a weak alcoholic fermentation and a large amount of acid, which coagulated the milk.

(2) In 1890 Weigmann (21) isolated a lactose-fermenting Saccharomycete from a sample of deteriorated butter.

(3) *Saccharomyces fragilis* n. sp. Jörgensen (12) was found in kefir; the cells of this form possessed a characteristic power of weak refraction of light. Alcohol and also acid were produced from lactose by this yeast.

(4) In 1897 von Freudenreich and Orla-Jensen (19) found a true Saccharomycete in their work on the influence of natural rennin upon the ripening of Swiss cheese. The authors could not identify this yeast as one of the described forms. The yeast was not found in the natural rennin, as it was destroyed by the heat used in the preparation, but was found to be present in the so-called "acid" used in making the rennin.

(5) In 1902 Orla-Jensen (16) found two lactose-fermenting true Saccharomycetes in his investigation on the production of rancidity in butter. These yeasts fermented maltose as well as lactose. Inoculated butter remained unchanged even after two months; the acid number did not increase, as the yeast lacked the power to split fat.

(6) In 1903 Macé (14) reported finding a lactose-fermenting true Saccharomycete in his work on the soft cheeses of France.

(7) In 1908 Weigmann, Gruber and Husz (22) reported a Saccharomycete, encountered in their work on Armenian matzun, which they called *Saccharomyces Pastorianus.*

(8) In the same year Kuntze (13) found a lactose-fermenting yeast, a true Saccharomycete, to be constantly present in yughourt, to which it gave, by reason of its "aroma building" power, a highly desirable ester-like flavor. Milk was fermented with the production of a fine foam and an aroma suggestive of dried fruit.

The original work conducted by Dombrowski (4) concerned itself mainly with cultures furnished by others. These had their origin in milk beverages, such as matzun, kefir, and yughourt, in kefir grains, in milk, butter and butter cultures. It was found that Torula species were much more commonly met with than true Saccharomycetes. The latter were divided as follows:

1. Genus Saccharomyces.
   a. *Sacch. lactis alpha.*
   b. *Sacch. lactis beta.*
   c. *Sacch. lactis gamma.*
2. Genus Zygosaccharomyces, represented by one species,—*Z. lactis alpha.*

Out of six cultures of true Saccharomyces worked with, four (comprising the alpha and beta species) fermented dextrose, galactose, lactose and sucrose, but not maltose. The other two cultures (*Sacch. lactis gamma*) fermented all the sugars but maltose and lactose. The one species of Zygosaccharomyces was isolated from butter, and was found to ferment dextrose, galactose, lactose, and sucrose, but not maltose, producing besides alcohol and

CO_2, a small amount of acid. This species showed a fusion of the cells before spore production. It was supposed to be the first lactose-fermenting yeast of the genus to be described.

Of thirteen cultures of torula forms worked with by Dombrowski, six fermented lactose with the production of CO_2, and the others fermented no sugars. These torula forms were isolated from matzun, kefir, and kefir grains, milk and butter.

Rubinsky (17) described a lactose-fermenting yeast which he isolated from kumiss. Spore production was not certain. Milk held at 37° C. for several days coagulated, following an evolution of CO_2. This coagulation was attributed to an enzyme. The yeast was supposed to differ from *Saccharomyces kefir* Beyerinck in its ability to coagulate milk and produce peptone.

EXPERIMENTAL

METHODS USED

The samples of milk, cream, etc., were examined for lactose-fermenting yeasts by plating on whey agar and incubating at 37° C., 1 cc. of a 1 per cent solution of tartaric acid being added to each plate just before pouring. Yeast colonies are easily detected on such plates and the low power of the microscope affords a simple means of making a decision in case of doubt. The different types of yeast colonies which developed on the plates were inoculated into milk and all those developing gas were reserved for study.

Enrichment methods were commonly used, especially if difficulty was encountered in securing a lactose-fermenting yeast from the material under study. These methods consisted of one or more transfers in milk or lactose bouillon, incubation being at 37° C.; plating was then carried out in the usual way.

Purification was accomplished by repeated plating. In some instances difficulty was experienced in securing pure cultures and bacteria were still present after several platings; the almost constant presence of bacteria in cultures of certain yeasts suggested a tendency of these organisms to attach themselves to the yeast cells.

The acidity of fermented milk was determined by titrating a 5 or 10 cc. portion with N/10 NaOH, using phenolphthalein as an indicator; the results were expressed as the percentage of lactic acid. If there was active gas evolution a 10 gram sample was used.

The amount of alcohol produced was estimated by distilling, after the addition of an excess of $CaCO_3$, and determining the specific gravity of the distillate. NaCl was usually added in considerable quantities and foaming was controlled with tannic acid.

Spore production was determined by the gypsum block method. After a number of transfers in glucose bouillon or on whey agar the cells were washed twice with sterile distilled water, using a centrifuge, and put on blocks; stains for spores were made after varying periods. Old agar cultures (from one to four months old) were also examined for spores, since tests with known spore formers showed that spores were readily detected in such cultures; if results are not wanted quickly this method has the advantage of involving much less work than the block method.

Giant colonies were secured by putting a small drop of a sugar bouillon culture on a whey gelatine plate and incubating at 20° C.

RESULTS SECURED

Samples of "yeasty" or "foamy" cream examined.

Twenty-one samples of definitely "yeasty" cream were examined; some of these were collected by a laboratory representative, while others were sent in on request, so the decision as to whether or not the samples were "yeasty" rested with a number of different people. The material was intentionally secured from rather widely separated sections so as to represent variable conditions. The number of samples of "yeasty" cream from each state was as follows: Iowa, 13; Kansas, 4; Missouri, 2; Illinois, 1; and Nebraska, 1.

From each of the 21 samples, a lactose-fermenting yeast was readily isolated without the use of enrichment methods. The organisms secured from twenty of the samples were of the same general type as regards morphology and the common growth characteristics; this type is referred to as Type A. Another type of organism, Type B, was secured from one of the samples from which Type A could not be isolated. In all of the samples of "yeasty" cream examined, the lactose-fermenting yeasts were present in considerable numbers.

Samples of number 2 cream examined.

Nine samples of number 2 cream were secured from Iowa creameries and examined for lactose-fermenting yeasts. The samples were taken from lots of cream that had been graded out by the cream graders and represented cream of a very poor quality. None of the samples showed any evidence of a "yeasty" condition.

Lactose-fermenting yeasts were isolated from eight (89 per cent) of the nine samples examined; from seven of the eight positive samples Type A was secured and the remaining one yielded Type B as the only lactose-fermenting yeast. The organisms were easily isolated, altho with a few of the samples enrichment methods were employed.

The results secured are of importance mainly in that they indicate that lactose-fermenting yeasts were commonly present in

samples of number 2 cream which showed no evidence of a "yeasty" condition.

Samples of clean-flavored sour cream examined.

Seventy-one samples of clean-flavored sour cream coming from various parts of Iowa were examined for the presence of lactose-fermenting yeasts. The common procedure was to hold the cream at 37° C. for from 24 to 48 hours and then plate in the usual way. With this method, yeasts were secured from all the samples examined, but only twelve of the 71 samples (17 percent) yielded lactose-fermenting types. Of the twelve positive samples, eleven yielded lactose-fermenters of the A type and one a lactose-fermenter of the B type.

The data presented show that lactose-fermenting yeasts were sometimes present in clean-flavored samples of sour cream, but that they were much less common than in the small number of samples of number 2 cream examined.

Presence of lactose-fermenting yeasts in milk.

Lactose-fermenting yeasts have been frequently met with in samples of milk from various parts of Iowa held at 37° C. for the purpose of isolating other types of organisms. When the milk was held in a tightly corked bottle it was very common to find the cork blown out and in some instances the bottle broken. No data were secured on the percentage of the samples of milk containing lactose-fermenting yeasts.

Lactose-fermenting yeasts were isolated from two samples of Iowa milk and also from a sample of milk sent from Denmark; these were of the A type. The isolations from milk were made by plating the milk after it had shown gas development.

A lactose-fermenting yeast of the A type was also secured from a sample of matzun. Altho the matzun had been carried for a considerable time in the United States, the original material was brought from Armenia; it is of course impossible to tell whether or not the yeast was a contamination picked up in this country.

STUDY OF THE LACTOSE-FERMENTING YEASTS

The 45 lactose-fermenting yeasts isolated from the samples of cream, both "yeasty" and not "yeasty," and from the samples of milk, were studied for the purpose of comparing them. The sources of these cultures are summarized in table I.

A careful study of the morphological, cultural and bio-chemical features of the 45 cultures led to their division into two types, Type A and Type B, as already mentioned, that are apparently quite distinct. Altho only three cultures of Type B were studied, because it is apparently rather unusual, these three cultures were so alike and so definitely different from the other cultures that their separation seemed justifiable. The main differences between the two types were as follows:

TABLE I—SOURCES OF LACTOSE-FERMENTING YEASTS STUDIED

Material used for Isolation	Origin by States and Countries	No. of Cultures
"Yeasty" cream	Ia., Kan., Mo., Ill., Neb.	21
Number 2 cream	Ia.	8
Clean-flavored sour cream	Ia.	12
Milk (including matzun)	Ia., Denmark, possibly Armenia	4
		Total 45

1. Type A was consistently larger than Type B.

2. Type A was oval or elliptical with elongated cells frequently present in certain cultures, while Type B, in the few cultures studied was always almost spherical.

3. In general Type A was a more rapid grower than Type B. While the optimum temperature for development of Type A was about 37° C. and that for Type B about 30° C., whey agar slope cultures of the former showed better development at 37° C. than did those of the latter at 30° C. Even when compared at the optimum temperature for Type B, whey agar slope cultures showed Type A to develop slightly better. At 37° C. the better development of Type A was marked. The same relationship was found in a comparison of whey agar plate colonies. After 24 hours at 37° C., Type A developed colonies about 2.5 mm. in diameter, while Type B, at its optimum temperature of 30° C., did not exhibit colonies of this size until after 48 hours. Type A showed a better development of colonies than Type B even at the optimum temperature of the latter. The more rapid growth of Type A was also shown by the rate of gas formation in various media containing a fermentable carbohydrate. The gas formed by a number of cultures in fermentation tubes containing sterile milk and whey after varying periods at 37° C. is shown in table II. The more rapid growth of Type A in these trials was due to the two factors mentioned above—first, the optimum for Type B was at a lower temperature than that for Type A, and second, Type A was in general a more rapid grower than Type B, even when both organisms were close to their optima.

4. All of the three cultures of Type B studied were true gelatine liquefiers, while true liquefaction was rare with Type A. The most of the cultures of Type A, however, changed the gelatine to a liquid condition at 20° C. but solidification took place at somewhat lower temperatures. When a series of Type A cultures was cooled below 20° C., a setting of the gelatine occurred first in one tube and then in another, until at about 8° C. most of them were solid. Cultures solidifying when cooled to about 8° C. were not considered true liquefiers, since cultures of Type B and known gelatine liquefiers did not do this. The reason for the peculiar behavior of cultures of Type A was at first thought to be due to the presence of alcohol, which is said to influence

TABLE II—RATE OF GAS FORMATION BY TYPES A AND B IN STERILE MILK AND WHEY IN FERMENTATION TUBES AT 37° C.

MILK

Culture No.	Type	Percent Gas after Various Periods									
		31 hr.	40 hr.	45 hr.	64 hr.	73 hr.	88 hr.	101 hr.	120 hr.	136 hr.	161 hr.
115	A	10	20	42	72	75	80	82	max.	....	....
142	A	trace	35	45	68	85	90	max.	....	....	....
179	A	trace	95	100	....	....	....	....	....	....	....
182	A	0	40	78	90	100	....	....	....	....	....
69	B	0	0	2	90	100	....	....	....	....	....
168-2	B	0	0	0	51	61	90	100	....	....	....
174-1	B	0	0	trace	61	68	80	85	85	98	100

NEUTRAL WHEY

Culture No.	Type	Percent Gas after Various Periods							
		41 hr.	53 hr.	[illegible] hr.	73 hr.	88 hr.	113 hr.	136 hr.	168 hr.
22	A	0	trace	1	5	30	75	max.	....
120	A	0	20	56	70	78	84	max.	....
147	A	20	80	85	max.	....	....	....	....
179	A	20	54	82	92	100	....	....	....
69	B	0	0	0	0	70	80	100	....
168-2	B	0	0	0	0	14	38	48	max.
174-1	B	0	0	0	0	25	78	max.	....

the solidifying properties of gelatine, but the addition of alcohol to uninoculated gelatine tubes failed to reproduce the condition.

5. Types A and B differed in their ability to ferment various materials. Both fermented fructose, galactose, glucose, lactose, and sucrose, but neither fermented glycerol, maltose, dulcitol, mannitol, or salicin. Type A usually fermented inulin and raffinose, while the three cultures of Type B fermented raffinose, but not inulin. The differences in the fermenting powers of the two types are shown in the following summary.

I. Fermenting inulin.
  a. Fermenting raffinose. — 37 cultures Type A
  b. Not fermenting raffinose. — 3 cultures Type A

II. Not fermenting inulin.
  a. Fermenting raffinose. — 3 cultures Type B
  b. Not fermenting raffinose. — 2 cultures Type A

6. Type B was not able to grow at 43° C., while some cultures of Type A grew at this temperature and some did not.

7. Some cultures of Type A exhibited a "radial thread" sub-surface colony in whey agar plates, somewhat similar in appearance to a mold colony. Type B showed only dense sub-surface colonies, with entire, well-defined edges.

Designation of the organisms studied.

The two types of lactose-fermenting yeasts were studied morphologically, culturally and bio-chemically and the descriptions, which are given below, have been compared with those of the torulae reported as having been isolated from milk and other dairy products; both the original reports and descriptions given

by Guilliermond (7) have been used. Many of the organisms have been so inadequately described that it is entirely impossible to say whether or not the organisms studied are like them. The problem is further complicated by the fact that the lactose-fermenters often coagulated milk very slowly and it is likely that organisms have been erroneously reported as not coagulating milk because no particular attention was given to this point. An attempt was made to use the names of organisms already described for the types studied, but this was found to be impossible, since the named organisms that were the most like these always showed differences that it seemed could not logically be disregarded. Accordingly, *Torula cremoris* is proposed for Type A and *Torula sphaerica* for Type B.

DESCRIPTION OF TYPE A — TORULA CREMORIS

MORPHOLOGY.

Form. In general the cells were oval or elliptical. Some cultures exhibited cylindrical and elongated cells; there was a certain variation in all cultures, but it was more pronounced in some than in others.

Size. In 24 hour whey agar slope cultures the organisms varied generally from about 2.5 to 3.6 microns in width and from about 4.1 to 6.1 microns in length. Some cultures showed elongated cells measuring usually about 3 to 4 by 13 to 14 microns altho cells as long as 19 microns were not uncommon.

Arrangement. The organisms commonly occurred singly; in young cultures a single bud was usually attached to the mother cell.

Spore Formation. The organisms were not observed to form spores, altho the most favorable conditions known for spore formation were employed.

Staining Reactions. The cells stained readily with the ordinary stains. They were Gram positive in young cultures, but in old cultures some Gram negative cells were observed.

CULTURAL CHARACTERISTICS.

Whey Agar Slope. After 24 hours at 37° C., a good development was evident. The growth was generally raised, glistening, non-viscous, whitish, smooth and opaque, the edge being entire, or slightly undulate. With age the amount of growth increased but the general character was retained. At room temperature the same type of growth was secured, but the development was not so rapid.

Beef Infusion Agar Slope. Growth was slow, only fair in amount, and apparently slightly better at 20° C. than at 37° C. It resembled in general characteristics that secured on whey agar.

Whey Agar Stab. After 24 hours at 37° C. there was a very heavy, white, glistening, non-viscous surface growth considerably raised and with an entire edge. There was a heavy growth all along the line of inoculation, altho development was best near the surface. After several days some cultures exhibited a suggestion of a villous character of growth, but with very short, thread-like projections along the stab. At room temperature the growth was of essentially the same character as at 37° C., but was less rapid.

Beef Infusion Agar Stab. Growth was slow and never abundant, but resembled in general characteristics the growth secured on whey agar.

Whey Agar Plate Colony. Colonies developed rapidly at 37° C. After 24 hours the surface colonies were usually large, averaging about 2.5

mm. in diameter, regularly circular, glistening, non-viscous, whitish, convex, smooth, and opaque, with an entire edge. Under the microscope the surface colonies appeared dense, with the typical edge of closely packed yeast cells. With some cultures the sub-surface colonies were consistently of the "radial thread" type, presenting an appearance similar to the hyphae of a mold colony; with other cultures they were dense, oval, spherical, or "torpedo-like," exhibiting an entire, definite edge. There were all gradations evident between these two extreme types of sub-surface colonies, but individual cultures were constant in the type of sub-surface colony developed. Growth at room temperature was the same as at 37° C. but was slower.

Giant Colony. After 18 days on whey gelatine, giant colonies measured usually about 9.5 to 13 mm. in diameter, were grayish, raised, slightly glistening, opaque, with a depressed, smooth center surrounded by a raised ring, from which radial contours extended to the edge. A softening of the gelatine took place usually after a number of days, the colonies sinking into the medium.

Whey Gelatine Slope. Growth was rather slow, but after six days a fair amount was present; it was filiform, whitish gray to cream colored, dull or slightly glistening, somewhat raised, with transverse contours, while the edge was straight or undulate. In some cultures a softening of the gelatine occurred in about two weeks.

Whey Gelatine Stab. Growth was slow, but developed well. After six days there was a fair amount of surface growth that was raised, circular, whitish gray to cream colored, dull, or slightly glistening, with radial contours. Along the line of the stab the growth was especially heavy just below the surface. After from two weeks to a month, a softening of the gelatine took place in some cultures, a few showed a true liquefaction, while still others exhibited no change of this nature, even when held 79 days after inoculation.

Bouillons. Growth in bouillons was evident in 24 hours at 37° C. as a whitish sediment. It was best in bouillons to which had been added materials that were fermented with the production of gas, but also occurred in plain bouillon and bouillons with non-fermentable materials. Surface growth was never observed. At room temperature growth was slower than at 37° C.

Potato. A luxuriant growth, varying in character slightly with the moisture content of the potato, was present after 24 hours at 20° C. In general the growth was slightly raised, whitish, glistening, and smooth.

Dunham's Solution. Growth was evident as a whitish sediment after several days at 37° C.

Uschinsky's Solution. Growth was evident as a whitish sediment after several days at 37° C.

Litmus Milk. After 24 hours at 37° C. much gas was formed and a small amount of acid; the litmus turned a definite pink thruout the tube. Coagulation took place after a number of days thru the agency of a rennin-like enzyme.

Plain Milk. After 24 hours at 37° C. gas evolution was pronounced. Coagulation took place after a number of days at 37° C.

BIO-CHEMICAL FEATURES.

Gas Production. Gas was produced in milk and in bouillons containing fructose, galactose, glucose, lactose and sucrose. The great majority of the cultures worked with also produced gas in inulin and raffinose bouillons, but three cultures failed to ferment raffinose and two did not ferment either inulin or raffinose. Gas production varied in amount with individual cultures, some fermenting only weakly. Bouillons containing

glycerol, maltose, dulcitol, mannitol, or salicin were not fermented with gas evolution by any of the cultures. Beer wort was commonly fermented with gas production, due probably to the presence of glucose.

Reaction Change. Sugar bouillons in which gas was produced were slightly acid in reaction, showing, on an average, from 1.6 to 1.9 per cent N/1 acid. A small amount of acid was also produced in milk.

Odor. Milk and sugar bouillons fermented with gas production gave a marked odor of alcohol, the odor being especially noticeable in milk. Agar plates emitted a pleasing, fruity odor.

Alcohol Production. In milk incubated at 37° C. for several days, alcohol was produced to the extent of 1.5 to 2.0 percent by weight.

Optimum Temperature. The optimum temperature for growth was found to be about 37° C.

Oxygen Relation. The organism was facultative, but grew best in the presence of a plentiful supply of oxygen.

DESCRIPTION OF TYPE B — TORULA SPHAERICA

MORPHOLOGY.

Form. The cells were generally almost spherical; definitely elongated cells were never observed.

Size. In 24 hour whey agar slope cultures, the cells averaged from about 3.0 to 3.1 by 3.0 to 3.3 microns, most of them being almost spherical.

Arrangement. The organisms commonly occurred singly, or with a single bud attached.

Spore Formation. Spores were not observed, even under the most favorable conditions known for spore formation.

Staining Reactions. The cells stained readily with the ordinary stains. In young cultures the organisms were Gram positive, but in old cultures some Gram negative cells were seen.

CULTURAL CHARACTERISTICS.

Whey Agar Slope. After 24 hours at 37° C., a raised, glistening, whitish, smooth growth, moderate in amount, was evident; the edge was entire or slightly undulate. At 30° C. and at room temperature the growth was of the same type, but development was somewhat faster than at 37° C.

Beef Infusion Agar Slope. Growth was generally slow and only fair in amount, but possessed the same general characteristics as that on whey agar. Development was better at room temperature and 30° C. than at 37° C.

Whey Agar Stab. In 24 hours at 37° C. a fair amount of surface growth was present, which was circular, slightly convex, whitish, glistening, smooth and opaque, with an entire edge. Along the line of inoculation a heavy filiform growth occurred that was best near the surface.

Beef Infusion Agar Stab. Growth was slow and never heavy but resembled in its general characteristics that secured on whey agar.

Whey Agar Plate Colony. In 24 hours at 37° C. the colonies were barely, if at all, visible. Plates held at 30° C. for the same length of time showed rather small surface colonies; in 48 hours these developed into colonies about 2.0 to 2.5 mm. in diameter that were regularly circular, whitish, glistening, convex, smooth, and opaque, with an entire edge. After 48 hours at 37° C. the colonies were definite but very small. Subsurface colonies always had an entire edge, and were generally spherical or oval in shape; no radiating filaments were ever present in the subsurface colonies.

Giant Colony. After 18 days on whey gelatine, giant colonies measured from 10 to 12 mm. in diameter and were generally yellowish or whitish,

raised, and dull. The center appeared deeply depressed and there were fine radial contours extending from the raised ring surrounding the center to the edge. The colonies were in general smooth, with entire, regular edges.

Whey Gelatine Slope. In six days a filiform growth, whitish gray to light cream in color, was evident. A uniform depression, like a furrow, extended in the center along the length of the streak. The growth was raised and slightly glistening, some of the cultures exhibiting cross contours while others were smooth. A softening of the gelatine began in some cases in fourteen days.

Whey Gelatine Stab. In six days a good amount of surface growth was evident; it was whitish gray or cream colored, smooth, slightly raised, glistening, and had an entire edge and radial contours. Along the line of the stab the growth was filiform and heaviest just below the surface. True liquefaction took place in about one month, but before this the cultures passed thru a softening stage in which solidification took place upon cooling to 8° C.

Bouillons. Growth in bouillons was evident as a whitish sediment in from 24 to 48 hours at 37° C. It was best in bouillons to which had been added materials that were fermented with the production of gas, but also occurred in plain bouillon and bouillons containing non-fermentable materials. Surface growth did not occur. Growth was more rapid at 30° C. than at 37° C.

Potato. A heavy growth, varying somewhat in character with the amount of moisture in the potato, took place after 24 hours at 20° C. Usually the growth was whitish, glistening, smooth, and only very slightly raised.

Dunham's Solution. Growth was evident as a whitish sediment after several days at 37° C.

Uschinsky's Solution. Growth was evident as a whitish sediment after several days at 37° C.

Litmus Milk. Very slight gas production occurred in 24 hours at 37° C.; a small amount of acid was produced, the litmus turning pink thruout the tube. Gas production was more marked at 30° C. Coagulation took place after a number of days at 37° C. thru the agency of a rennin-like enzyme.

Plain Milk. Very slight gas production occurred in 24 hours at 37° C. Coagulation occurred after a number of days at this temperature.

BIO-CHEMICAL FEATURES.

Gas Production. Gas was produced in milk and in bouillons containing fructose, galactose, glucose, lactose, sucrose, and raffinose. Gas production was stronger with some of these materials than with others. None of the cultures was able to produce gas in bouillons containing glycerol, maltose, dulcitol, mannitol, salicin, or inulin.

Reaction Change. Sugar bouillons in which gas was produced were slightly acid in reaction, showing on an average, from 1.4 to 1.8 per cent N/1 acid. A small amount of acid was also produced in milk.

Odor. Agar plates emitted a pleasant, fruity odor. In milk and sugar bouillons fermented with gas production there was a marked odor of alcohol.

Alcohol Production. In milk incubated at 37° C. for a number of days, about 1.8 percent of alcohol by weight was produced.

Optimum Temperature. The optimum temperature for growth was found to be about 30° C.

Oxygen Relation. The organism was facultative, but grew best in the presence of an abundant supply of oxygen.

TABLE III—ACIDITIES (CALCULATED AS LACTIC ACID) IN SKIM MILK AT THE TIME OF COAGULATION

Culture No.	Type	Temp. of Incubation	Days to Coagulate	Acidity
69	B	37° C.	7	0.31%
174-1	B	37° C.	11	.40
174-1	B	30° C.	24	.23
168-2	B	30° C.	27	.34
147	A	43° C.	14	.33
147	A	20° C.	58	.38
39	A	37° C.	4	.31
176	A	37° C.	4	.27
179	A	37° C.	4	.36
182	A	37° C.	4	.26

TABLE IV—ACIDITIES (CALCULATED AS LACTIC ACID) IN STERILE SKIM MILK AFTER VARYING PERIODS AT DIFFERENT TEMPERATURES

Culture No.	Type	Temperature	24 hrs.	48 hrs.	3 days	4 days	5 days	7 days	8 days	14 days	21 days
22	A	37° C.		.50%		.48%		.41%			
28	A	20° C.			.55%		.54%		.50%	.34%	
		25° C.			.52		.50		.37	.38	
		37° C.		.50		.42		.38			
39	A	20° C.	.21%		.45		.58		.36	.32	.35
		37° C.		.47		.34		.42			
43	A	20° C.			.39		.59		.42	.35	.36
		37° C.		.51		.35		.38			
47	A	20° C.	.23		.49		.56		.44	.37	.39
69	B	20° C.		.46	.52		.50		.40	.35	.36
		25° C.			.57		.50		.38	.33	
		37° C.		.45		.39		.44			
70	A	20° C.			.49		.54		.43	.32	.37
		37° C.		.50		.43		.37			
80	A	20° C.			.50		.56		.42	.36	.40
		37° C.		.50		.36		.39			
120	A	20° C.	.24		.45		.52		.55	.37	.39
		25° C.			.46		.50		.35	.37	
		37° C.		.48		.50		.39			
143	A	20° C.	.28		.54		.55		.42		
		37° C.		.52		.49		.38			
147	A	20° C.	.23		.49		.59		.36	.35	.35
		25° C.			.50		.54		.36	.34	
		37° C.		.55		.47		.37			
158	A	20° C.			.48		.55		.49	.37	
		25° C.			.52		.53		.34	.35	
		37° C.		.52		.37		.38			
159	A	20° C.			.40		.54		.48	.36	.36
		25° C.			.56		.47		.33	.33	
		37° C.		.46		.42		.38			
168-2	B	20° C.			.50		.56		.42	.36	.37
		25° C.			.53		.53		.34	.31	
		37° C.		.37		.38		.40			
170	A	20° C.		.42	.47		.52		.48	.34	.32
		37° C.		.49		.50		.40			
174-1	B	20° C.			.47		.59		.50	.39	.41
		25° C.			.45		.54		.33	.29	
176	A	37° C.		.51		.48		.40			
177	A	37° C.		.47		.36		.37			
179	A	37° C.		.48		.40		.42			
183	A	37° C.		.48		.35		.38			

ADDITIONAL DESCRIPTION OF CHARACTERISTICS OF YEASTS ISOLATED

All of the lactose-fermenting yeasts studied curdled milk and the coagulation was not due to the acid, as is shown by table III, which gives the acidities present in milk at the time of coagulation after inoculation with various cultures. The data show that coagulation occurred at acidities varying from 0.23 to 0.40 per cent and accordingly it must be assumed that a rennin-like enzyme was responsible for the curdling.

Higher acidities were observed soon after inoculation than later on, as is shown in table IV; this was undoubtedly due to the presence of much larger quantities of CO_2 during the period of active gas formation than later when gas evolution was no longer evident. Table V gives the final acidities produced by various milk cultures and the results presented show that the final acidity reached by most cultures was not sufficient to cause the coagulation of milk.

The rate of coagulation was materially influenced by various conditions. A temperature of 37° C. was satisfactory and coagulation took place here much more rapidly than at room temperature, 30° C., or 43° C.; table VI gives results showing, in a general way, the length of time required for curdling at different temperatures. At all the temperatures tried there was a great deal of variation in the time required for curdling by different cultures and also by the same culture in different tests.

TABLE V—MAXIMUM ACIDITY (CALCULATED AS LACTIC ACID) PRODUCED IN STERILE SKIM MILK

Culture No.	Type	Incubation Days at 37° C.	Acidity	Days to Coagulate
22	A	33	.45%	5
39	A	28	.40	8
40	A	28	.42	3
42	A	28	.38	4
43	A	33	.43	5
44	A	28	.45	2
53	A	28	.43	3
60	A	28	.38	4
73	A	28	.54	5
80	A	28	.45	8
96	A	28	.44	3
105	A	33	.46	4
111	A	33	.45	4
115	A	33	.41	4
120	A	33	.50	4
143	A	28	.40	5
144	A	28	.42	5
147	A	33	.43	5
156	A	12	.41	3
157	A	28	.29	4
158	A	28	.43	3
159	A	28	.47	4
160	A	28	.40	4
162	A	28	.40	4
163-1	A	28	.34	4
165	A	28	.38	4
168-2	B	22	.51	29
169	A	28	.37	5
170	A	28	.40	5
171	A	28	.41	4
172	A	28	.41	4
173	A	28	.43	3
174-1	B	28	.52	12
175	A	28	.42	6
178	A	33	.68	4
179	A	28	.38	4
180	A	[illegible]	.36	4
181	A	28	.50	5
182	A	28	.50	4
183	A	28	.34	4
186	A	28	.42	4

TABLE VI—TIME REQUIRED TO COAGULATE MILK AT DIFFERENT TEMPERATURES

Culture No.	Type	Temperature	Days to Curdle	Temperature	Days to Curdle	Temperature	Days to Curdle
115	A	20° C.	24	37° C.	3	43° C.	8
147	A	20° C.	58	37° C.	6	43° C.	14
69	B	20° C.	32	37° C.	7	30° C.	17
168-2	B	20° C.	not in 28	37° C.	10	30° C.	27
174-1	B	20° C.	45	37° C.	6	30° C.	24
176	A	20° C.	not in 28	34° C.	12	38° C.	4
182	A	20° C.	not in 25	37° C.	4		
71	A	20° C.	not in 25	37° C.	5		

Coagulation of the milk took place more quickly in bottles or flasks than in test tubes. In some instances a culture failed to curdle a certain tube of litmus milk, altho it caused a vigorous gas development, but transfers made to other tubes of milk showed coagulation. The length of time required for coagulation in both flasks and tubes is shown for certain cultures in table VII; no explanation for the variations could be found, altho an attempt was made to work out an explanation on the basis of such factors as air supply, presence of soluble calcium salts and the extent of heating during sterilization. Coagulation generally occurred in a shorter time in whole milk in test tubes than in skim milk in the same kind of containers. It seems probable that the long periods required for coagulation in test tubes and the actual failures to secure such a change in some cases are the reasons why certain investigators have not reported the cultures of yeasts they studied as capable of coagulating milk.

TABLE VII—COMPARISON OF FLASKS WITH TUBES IN TIME REQUIRED TO CURDLE SKIM MILK AT 37° C.

Culture No.	Type	Number Days Required to Curdle		Culture No.	Type	Number Days Required to Curdle	
		In Flasks	In Test-tubes			In Flasks	In Test-tubes
22	A	5	12	143	A	5	26
39	A	8	14	147	A	5	11
40	A	2	26	157	A	4	11
43	A	5	16	158	A	3	16
44	A	2	14	159	A	4	11
53	A	8	14	160	A	4	16
70	A	4	16	162	A	4	22
73	A	5	14	163-1	A	4	22
80	A	8	19	170	A	5	11
96	A	2	6	180	A	4	16
111	A	4	20	182	A	4	22
115	A	4	15	186	A	4	23

From a number of experiments carried out it seems that the coagulating enzyme, while apparently produced in considerable amounts at temperatures such as 20° C., is much more active at higher temperatures. Cultures held for long periods of time at 20° C. without coagulating, showed this change in a comparatively short time at higher temperatures. For example, a culture which had been held at 20° C. for 28 days without coagulation, showed this change in 70 minutes after it had been placed at 43° C. In another instance a culture, after being held at 20° C. for 56 days without showing coagulation, showed this change in a portion of the culture held at 43° C. in about the length of time required to thoroly warm it up, while the remainder of the culture at 20° C. first showed coagulation after 58 days. Cultures held for long periods at 20° C. without coagulation showed this change when placed at 37° C., but not as quickly as at 43° C.

The alcohol produced by the lactose-fermenting yeasts was never very high in milk, the maximum percentage found being 2.28 percent by weight. Table VIII presents data showing in a general way the amount of alcohol produced in milk at 20° C., 25° C. and 37° C.; the results show that alcohol production occurred at all the three temperatures tried and that apparently 25° C. was very favorable.

Table IX gives the alcohol produced by the lactose-fermenting yeasts in bouillons containing glucose, lactose, and sucrose in 5 and 10 percent amounts, when incubated for 21 days at 37° C. The data show that under suitable conditions the yeasts can produce alcohol in excess of the amounts they produce in milk. The maximum percentage observed was 4.00 and this was produced in 10 percent lactose bouillon. The 10 percent sugar

TABLE VIII—ALCOHOL PRODUCTION IN STERILE SKIM MILK

Culture No.	Type	Time in Days	Temperature	Percent Alcohol by Weight
28	A	22	20° C.	1.94
28	A	33	25° C.	2.28
40	A	16	37° C.	1.37
163-1	A	19	20° C.	1.62
163-1	A	18	25° C.	2.28
163-1	A	24	37° C.	2.11
165	A	20	25° C.	1.75
165	A	13	37° C.	1.87
174-1	B	16	20° C.	1.50
174-1	B	16	25° C.	1.81
182	A	5	20° C.	1.06
182	A	5	25° C.	2.11
182	A	5	37° C.	2.00
182	A	11	20° C.	2.11
182	A	11	25° C.	1.81
182	A	11	37° C.	1.81

TABLE IX—ALCOHOL PRODUCTION IN SUGAR BOUILLONS
Incubation 21 days at 37° C.

Culture No.	Type	Sugar Bouillon Kind	Percent Sugar	Percent Alcohol by Weight
28	A	Sucrose	5	2.06
			10	3.82
42	A	Sucrose	5	1.87
			10	3.18
69	B	Sucrose	10	3.65
115	A	Lactose	5	1.94
147	A	Lactose	5	1.94
174-1	B	Lactose	5	1.56
158	A	Lactose	10	4.00
159	A	Lactose	10	3.71
186	A	Lactose	10	3.35
168-2	B	Glucose	10	3.24
181	A	Glucose	10	3.65

bouillons always gave alcohol in excess of that produced in those containing only 5 percent.

THE PRODUCTION OF TYPICAL "YEASTY" CREAM

Altho pure cultures of the lactose-fermenting yeasts produced much gas in milk, they did not produce a typical "yeasty" condition, in which the material continued for some time to increase in volume and a portion to be forced rather slowly from the container. The bubbles of gas passed rapidly through the milk and broke at the surface so that there was no definite and decided volume change, altho there was considerable foam. With cream, which has a greater viscosity than milk, the bubbles of gas seemed to be prevented from rapidly escaping, and in this material a condition quite like that of typical "foamy" cream could be produced. Milk which had been previously coagulated by the inoculation of *S. lacticus*, for the purpose of preventing the rapid escape of gas, did not exhibit the typical "foamy" character upon introduction of the lactose-fermenting yeast. While the yeast was capable of causing coagulation in milk, this occurred only after some time, when the rapid evolution of gas had ceased. Apparently the production of the typical "yeasty" condition in cream is very intimately connected with the prevention of the escape of the bubbles of gas formed. A "yeasty" cream was produced by the inoculation of a pure culture of the yeast into sterile cream, but apparently coagulated cream, upon introduction of the yeast, approached more closely the typical "foamy" condition so commonly met with in practice, since it continued to "boil over" for a longer period of time than did the uncoagulated cream. Accordingly it seems justifiable to assume that the condition of "foamy" cream as observed under practical conditions is not the result of the action of a lactose-fermenting yeast

alone, but is produced by a combination of the yeast and organisms which are capable of causing a rapid coagulation. It is probable that the decreased solubility of gas at higher temperatures is important, in that fermenting milk or cream, when raised somewhat in temperature, as is apt to occur during the shipment of cream in the warm summer months, produces bubbles of gas more rapidly than would be the case as a result of the immediate action of the yeasts. In inoculation experiments, the transfer of cream to 37° C. after a considerable period of incubation at a lower temperature often resulted in a "foaming-over" of the material in the container. It seems, therefore, that the temperature change may be important in the production of typical "foamy" cream.

In the attempts to produce "foamy" cream, better results were secured when the containers were corked, or in the case of a tin container, closed with a cover, than when they were stoppered with cotton or left open. It seems probable that the effect here was due to the influence on the amount of gas in solution; with the tightly closed containers there was a larger amount of gas held in the cream, so that when the stopper or cover was blown out or removed, the gas quickly formed bubbles, the volume increased, and the cream foamed over.

With gas-producing yeasts, the supersaturation with gas of the medium in which they are growing is very common. This gas is ordinarily in part quickly released on agitation. The agitation of cream inoculated with lactose-fermenting yeasts seemed to have an effect on the production of a typical "foamy" condition presumably due to the liberation of quantities of gas present in excess of the amounts required for saturation. During the shipment of cream in cans and during the initial handling at the manufacturing plant, the agitation would tend to release gas present in amounts in excess of that required for saturation.

DISCUSSION OF RESULTS

From the data presented it is evident that lactose-fermenting yeasts are readily isolated from samples of "yeasty" cream and that these are largely of one type. The results obtained also show that the same type of yeast is very common in number 2 cream and fairly common in clean-flavored sour cream, while the general statements made show it is also present in milk. Another type of lactose-fermenter possessing characteristics definite enough to distinguish it from the first, is apparently much less common, having been found in only one sample of "yeasty" cream, one sample of number 2 cream and one sample of clean-flavored sour cream.

It is probable that the more rapid growth and high optimum

temperature of the first type are the characteristics responsible for its much greater prevalence.

It is evident that the yeasts responsible for "yeasty" cream are much more common than the number of samples of cream developing a "yeasty" condition would indicate, and accordingly it seems probable that whether or not a sample of cream becomes "yeasty" is largely determined by the conditions under which the cream is held. The yeasts generally responsible for "yeasty" cream grow best at a fairly high temperature and it would be expected that the greatest trouble would be experienced during the warm months. Samples of milk and some few samples of cream have been put at 37° C. during the cold months and the development of gas by yeasts has been commonly observed; this indicates that the lactose-fermenters are also present in milk and cream during the colder weather, and in conformity with this an occasional batch of "yeasty" cream is encountered during the winter months, especially in lots that have been held at a fairly high temperature. The evidence seems to show that cream is quite likely to contain lactose-fermenting yeasts capable of producing a "yeasty" fermentation under the proper conditions, but that the conditions are not generally suitable for the fermentation to occur, altho they are much more commonly so in summer than in winter.

The production of a typical "yeasty" condition is, however, influenced by other factors than the presence of lactose-fermenting yeasts and a temperature suitable for their growth. It seems that among these additional factors those of the most importance are a viscosity, or thickening due to coagulation, sufficient to prevent too rapid an escape of the gas formed, the decreased solubility of the gas at a higher temperature, the closing of the containers, and agitation. A suitable balance between all or nearly all of the influencing factors is likely necessary before a typical "yeasty" condition results.

From the standpoint of the elimination of "yeasty" cream under practical conditions, the logical thing seems to be the prevention of the growth of the yeasts. Their frequent presence in milk and cream suggests a wide distribution of the organisms and under these conditions it is inadvisable to attempt to exclude them from cream. The next possibility is to prevent their development, and according to present knowledge, this can best be accomplished for the small shipper by the use of low temperatures. In the case of larger shipments, such as those coming from cream stations, where the volume of cream justifies the expense, effective pasteurization offers another method of preventing a loss of cream or a deterioration in quality. Yeasts are comparatively non-resistant to heat and pasteurization affords a satisfactory method of destroying them.

SUMMARY

1. Lactose-fermenting yeasts were isolated from all of 21 samples of "yeasty" cream secured from five states, from eight (89 percent) of nine samples of number 2 cream obtained from Iowa, from twelve (17 per cent) of 71 samples of clean-flavored sour cream coming from Iowa, from three samples of milk (two from Iowa and one from Denmark), and from one sample of matzun.

2. The 45 cultures studied were divided into two distinct types. These types did not agree closely enough with described organisms so that they could be considered like any of them and accordingly the names *Torula cremoris* and *Torula sphaerica* are proposed.

3. The data indicate that lactose-fermenting yeasts like those causing a "yeasty" condition in cream are much more common than the number of lots of cream developing "yeastiness" would indicate. It seems evident that the conditions to which cream is usually subjected are not suitable for the development of "foaminess," altho such conditions are much more commonly fulfilled in warm weather than in cold.

4. Factors other than the presence of lactose-fermenting yeasts and a temperature suitable for their growth that seem to be important in the development of a typical "yeasty" condition in cream, are a viscosity, or thickening due to coagulation, sufficient to prevent too rapid an escape of gas, a decreased solubility of the gas when the fermenting cream is placed at a higher temperature, the closing of the containers, and agitation.

5. Because of the wide distribution of lactose-fermenting yeasts, it does not seem advisable to attempt to keep them out of cream in order to prevent losses. The best thing that can be done at present by the small shipper is to hold cream at temperatures sufficiently low so that the development of the yeasts will be prevented or greatly retarded. Losses in large shipments of cream can undoubtedly be eliminated by efficient pasteurization and this may eventually prove to be a help in the solution of the "yeasty" cream problem.

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